Technical Explanation

US Application 10/583,706

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Claim 1 of the present application

(TP-FP primer set)

A primer set comprising at least two primers that allows a target nucleic acid sequence to be amplified,

wherein a **first primer** included in the primer set contains, in its 3' end portion, a sequence (Ac') that hybridizes to a sequence (A) located in the 3' end portion of the target nucleic acid sequence, and also contains, on the 5' side of the sequence (Ac'), a sequence (B') that hybridizes to a complementary sequence (Bc) to a sequence (B) that is present on the 5' side with respect to the sequence (A) in the target nucleic acid sequence, and

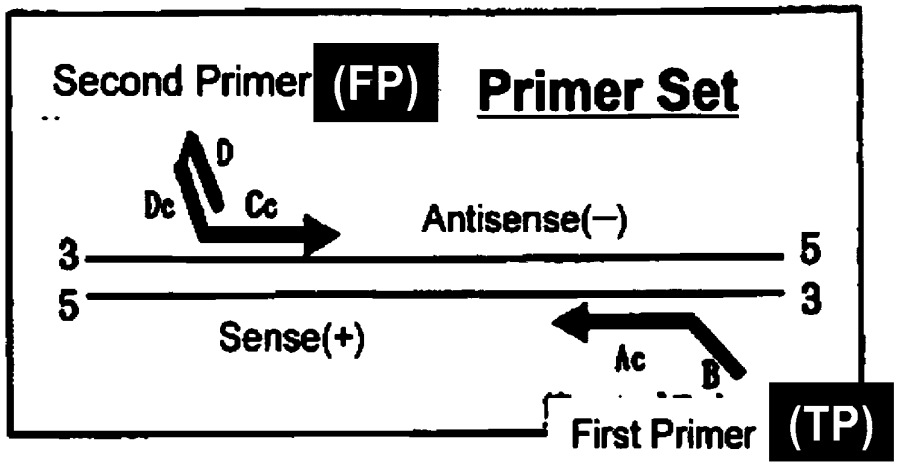
a **second primer** included in the primer set contains, in its 3' end portion, a sequence (Cc') that hybridizes to a sequence (C) located in the 3' end portion of a complementary sequence to the target nucleic acid sequence, and also contains, on the 5' side of the sequence (Cc'), a folded sequence (D-Dc') that contains, on the same strand, two nucleic acid sequences that hybridize to each other.

*The first primer is **TP**, the second primer is **FP**.

TP; Turn-back Primer

FP; Folded Primer

Technical explanation of the TP and FP

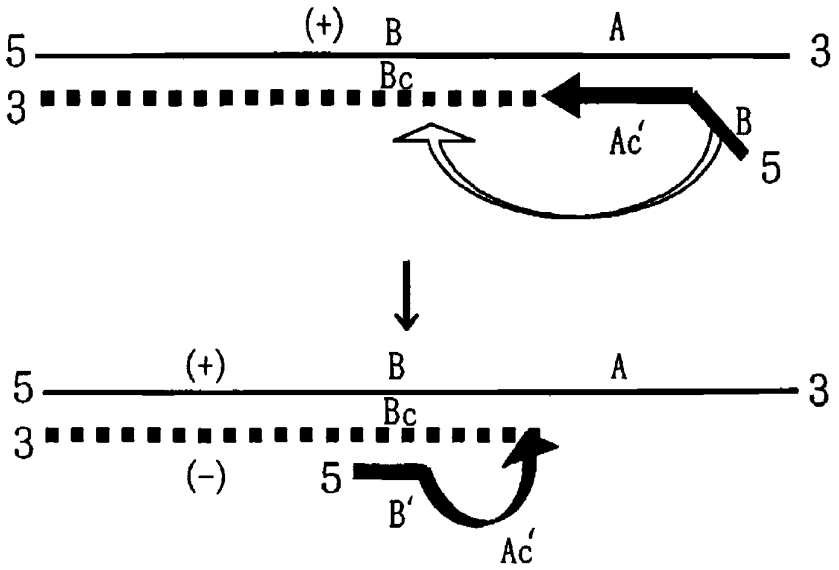


TP has the function as follows;

(1) TP has the turn back portion (B) in the 5' side sequence.
(2) The turn back portion (B) can hybridize to the portion (Bc) of the elongation strand from TP.

FP has the function as follows;

(1) FP has the folded sequence (D-Dc') in the 5' side sequence.
(2) The folded sequence (D-Dc') has two nucleic acid sequences that hybridize to each other.
(3) The folded sequence (D-Dc') **DO NOT** hybridize to the elongation strand from FP.



Mechanism of the amplification reaction of the TP-FP(1)

(FIG.3 of the present invention)

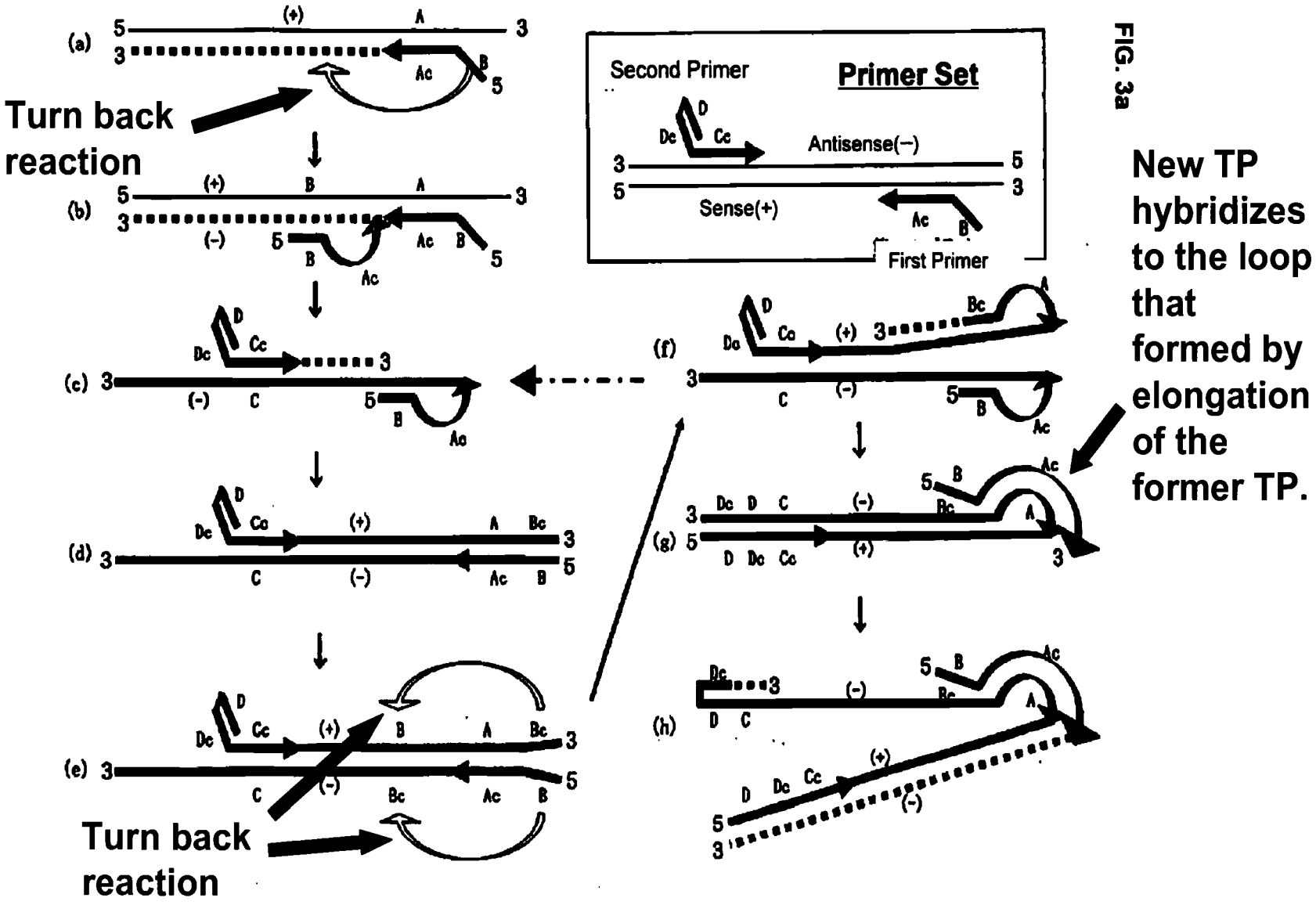


FIG. 3a

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Mechanism of the amplification reaction of the TP-FP(2)

(FIG.3 of the present invention)

TP **DOES NOT** hybridize to the folded portion formed by elongation of the former FP.

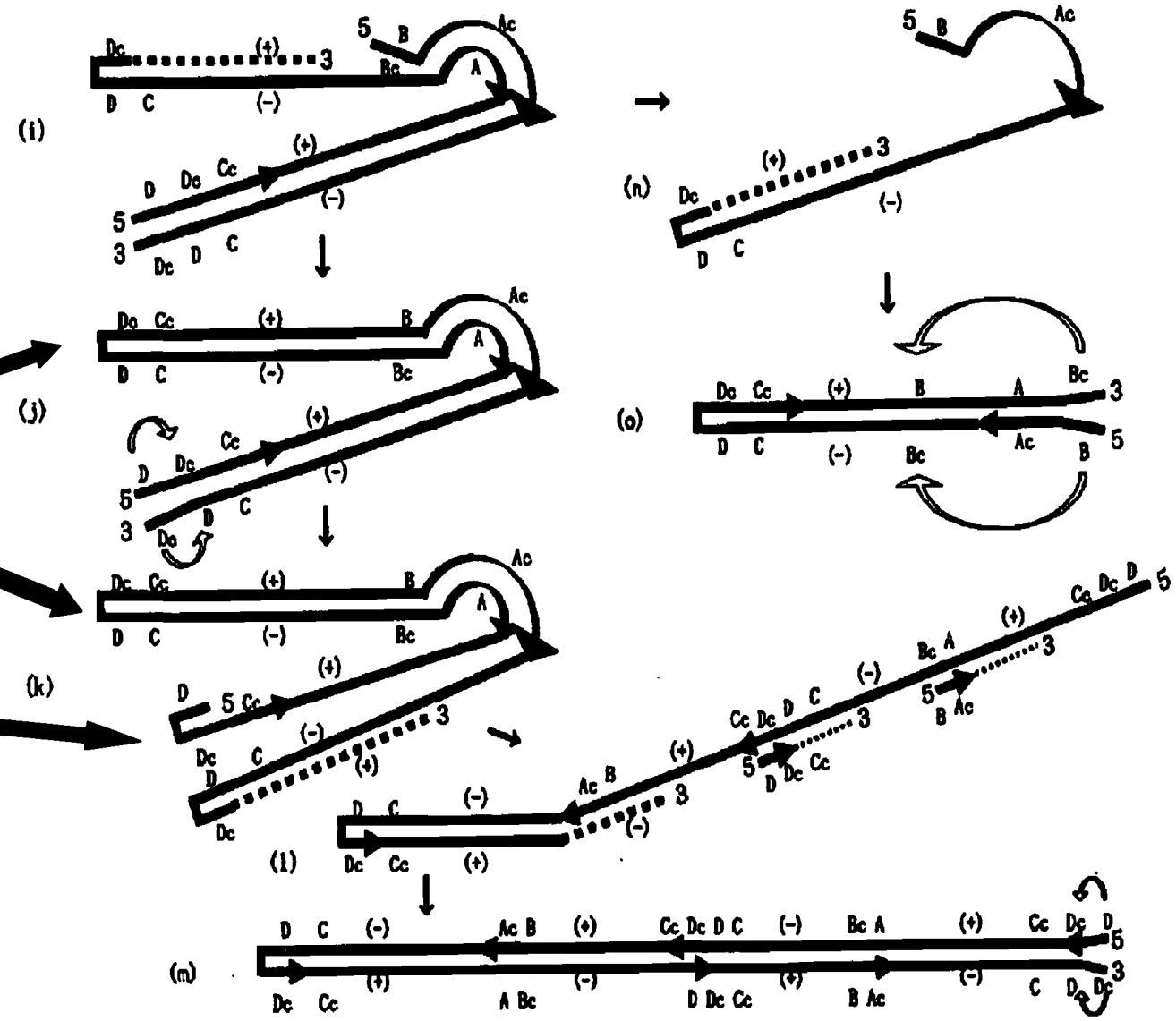


FIG. 3b

The present invention has four advantages.

(1) Isothermal amplification

- The amplification occurs without thermal denaturation.

(2) Specific amplification

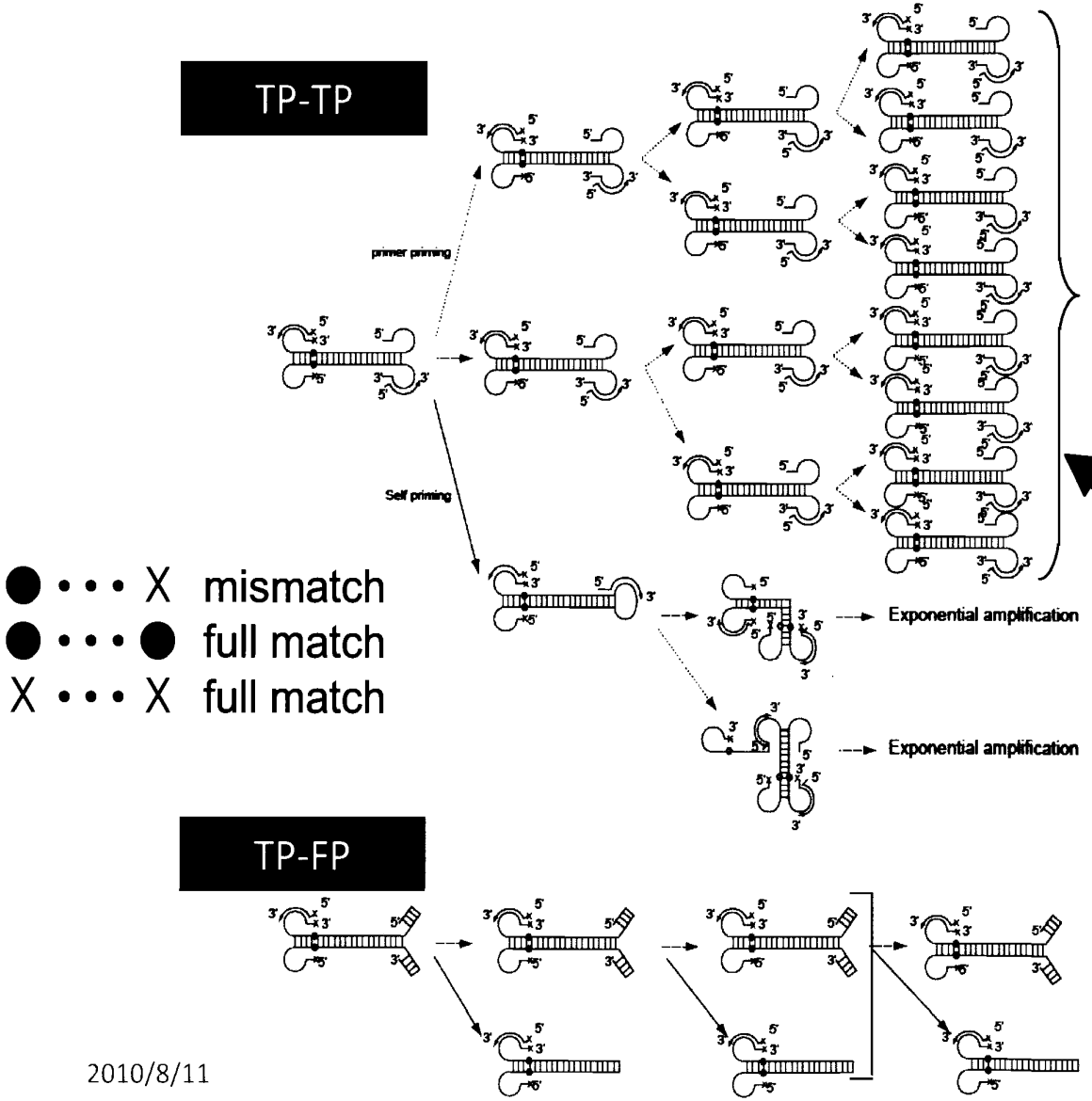
- The present invention can detect SNPs without non-specific amplification.

(3) Short time amplification

(4) Easy primer design

Mechanism of specific amplification

The non-specific amplification **DOES NOT** occur in the present invention (TP-FP).



(1) TP-TP primer set **CAN NOT** detect SNPs.
(2) Because, TP-TP primer set has the pathway of the non-specific extensively exponential amplification (background amplification).

Exponential amplification

(1) TP-FP primer set **CAN** detect SNPs.
(2) Because, the non-specific exponential amplification of the pathway of TP-FP primer set is very gentle.

Short time amplification and easy primer design(1)

(1) TP

(i) TP can amplify exponentially.

(ii) TP has a strong engine of amplification.

(iii) TP has two area depending on template sequence.

(2) FP

(i) FP can not amplify exponentially, but amplify linearly.

(ii) FP is like a mirror which reflect TP amplification.

(iii) FP needs only one area depending on template sequence.

(3) TP-TP primer set

(i) TP-TP primer set needs four areas depending on the template sequence.

(ii) TP-TP Primer set needs a design of a couple of good TP because the reaction is totally controlled by no good TP.

(iii) TP-TP Primer set is difficult to design.

(4) TP-FP primer set

(i) TP-FP primer set needs only three areas depending on the template sequence.

(ii) TP-FP Primer set needs a design of only one good TP because FP whose folded sequence can be designed in advance independently from template sequence **DOES NOT** control the reaction.

(iii) TP-FP Primer set is easy to design.

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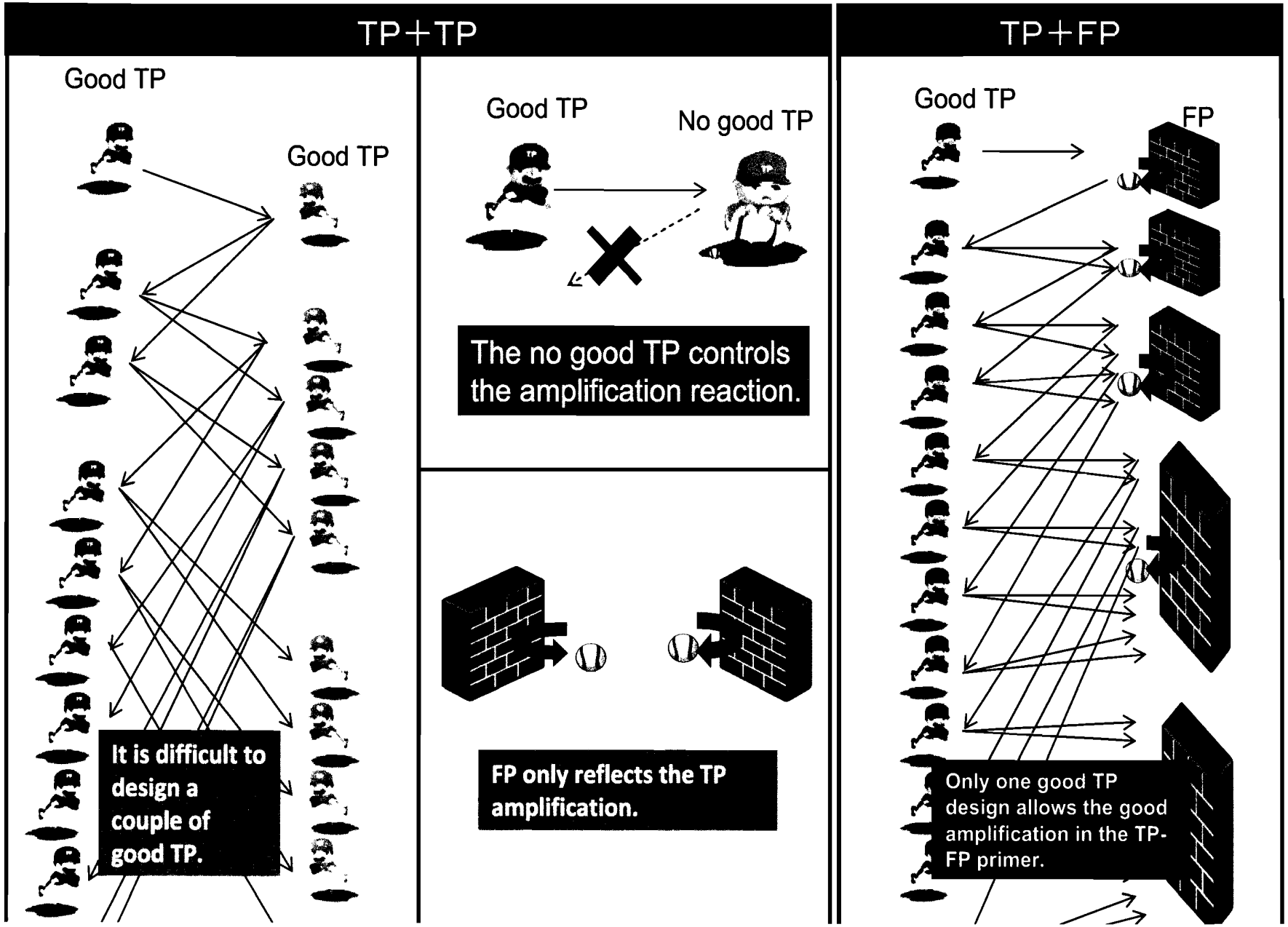
Short time amplification and easy primer design(2)



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Office Action (1)

· Summary of the Office Action

The examiner pointed out as follows;

(1) TP are shown in Figure 4, step 1 and 2 (① and ② shown in below left) in Rabbani (EP0971039A2).

(2) FP are shown in Figure 1, step 3 (③ shown in below right) in Rabbani.

(3) Therefore, Claims 1 to 5 of the present invention lacks novelty (102(b)).

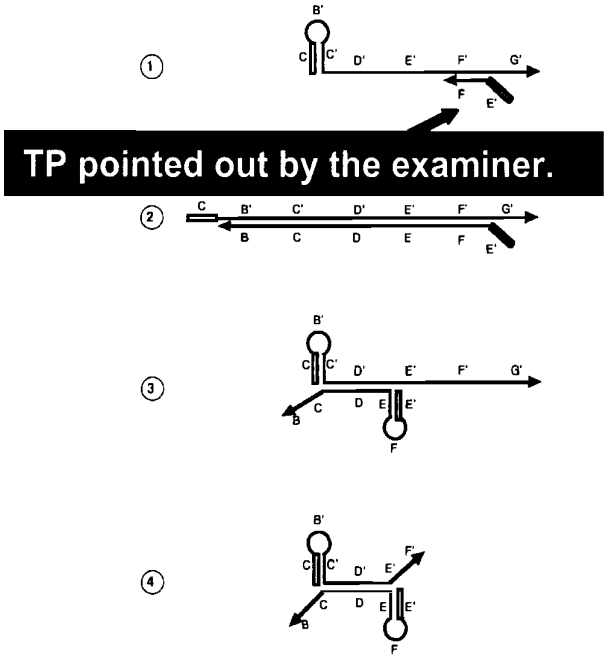


FIGURE 4

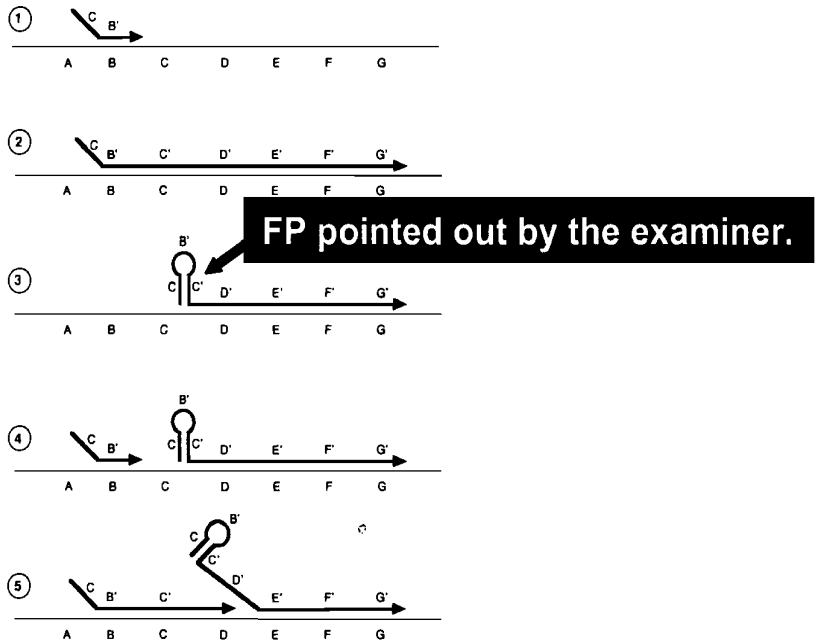


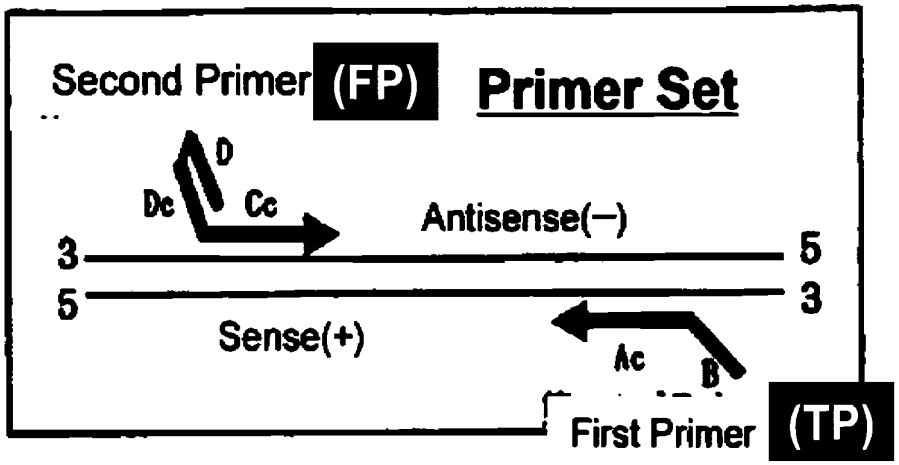
FIGURE 1

Office Action (2); FIGURE 1 ③ in Rabbani is NOT FP.

(1) FIGURE 1③ in Rabbani shows the elongation strand from TP.

(2) **Primer is different from the elongation strand.**

(3) Rabbani **DOES NOT** show the TP-FP primer set of the present invention.



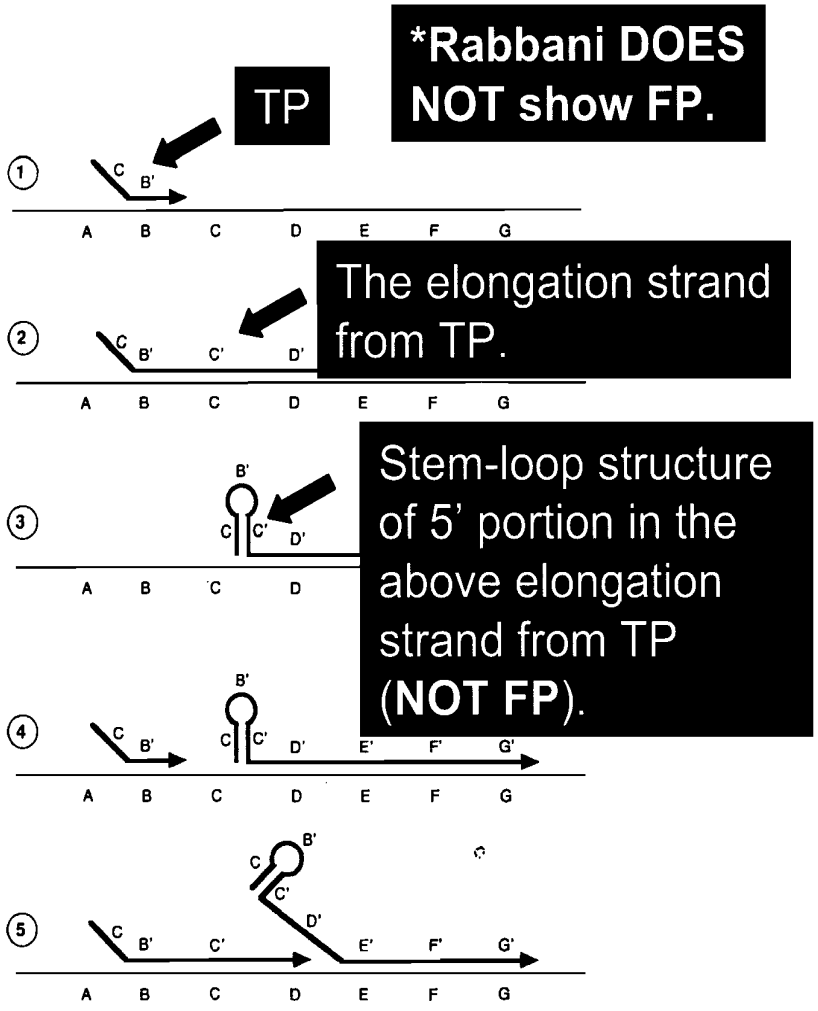


FIGURE 1

In case FP is used in the FIGURE 1 in Rabbani.